(presumably α_{1B} and α_{1L} subtypes), both of which are involved in the noradrenaline-induced contraction.

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MINOR ROLE FOR DIRECT ADRENOCEPTOR-MEDIATED CALCIUM ENTRY IN RAT MESENTERIC RESISTANCE ARTERIES

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Ca^{2+}-entry into vascular smooth muscle cells occurs through potential-dependent, dihydropyridine-sensitive calcium channels, and possibly also through calcium channels which are activated by receptor activation, independent of membrane potential (ROCs). We have investigated the possible functional role of adrenoceptor-mediated ROCs in rat mesenteric small arteries from Wistar rats. $[Ca^{2+}]_i$ determined by fura-2 fluorescence and force were measured simultaneously using a myograph. Depolarization with high-potassium solution (K-PSS) elevated $[Ca^{2+}]_i$ and induced contraction. Further addition of 10 µM noradrenaline (NA) did not elevate $[Ca^{2+}]_i$ further, but enhanced tone, thus confirming that agonist activation causes calcium-sensitization. When this protocol was repeated in the presence of calcium channel inhibitors (felodipine (1 nM) or D-600 (1 µM)), K-PSS induced neither maintained tone nor maintained elevation of $[Ca^{2+}]_i$, but the further addition of NA still caused a rise in tone and $[Ca^{2+}]_i$, to about half the previous levels. Repetition of the protocol in the presence both of calcium channel inhibitors and also drugs which deplete intracellular calcium stores (ryanodine (10 µM) or thapsigargin (1 µM)), showed that the NA-induced rise in $[Ca^{2+}]_i$ was inhibited, but a small (10-20% of control) rise in tone remained. The experiments suggest that ROCs do not contribute substantially to control of $[Ca^{2+}]_i$, and that effects of NA on tone which are independent of membrane potential are due primarily to calcium-sensitization but also to release of Ca^{2+} from intracellular stores.

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MODE OF EXISTENCE OF 74 KDA PROTEIN, ONE OF THE GELSOLIN FAMILY IN DEVELOPMENTAL STAGE OF VASCULAR SMOOTH MUSCLE

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The 74 KDa protein of the gelsolin family is distributed in non-muscle cells with exocytic function, but not in normal adult smooth muscle cells. In primary culture of smooth muscle from bovine carotid artery, we found that this protein was gradually expressed, arriving at maximum in confluent state by immunoblotting method. On the other hand, in the immunofluorescent method of the cultured cells 74 Kda protein was not expressed in all the cells with deformed globular feature on the 1st day without serum, but smooth muscle type of alpha-actin and smooth muscle myosin were clearly expressed. On 4th day after addition of serum, 74 KDa protein was expressed at the surface membrane of all the globular cells of which numbers were slightly increased by slow cell division. At time bipolar cells appeared underneath the globular cells. These cells had no 74 Kda protein and alpha-actin. Once bipolar cells appeared, their growth rate was very fast and their numbers increased rapidly. When cells were proceeding to confluent state, accompanied by hills and valleys structure, globular cells were gathered, and formed tight round structure on the top of the hills. At that time alpha-actin began to be expressed in abundant bipolar cells.

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CORONARY VASODILATOR RESPONSE TO BRADYKININ: ROLE OF CYTOCHROME P450 AND K^+ CONDUCTANCE

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The rat coronary vasodilator response to bradykinin (BK) is reportedly independent of NO and cyclooxygenase and lipoxygenase products of arachidonic acid (AA). We investigated the role of cytochrome P450 (P450) in the coronary vasodilator response to BK using the Langendorff heart preparation which was preconstricted with nitroarginine (50 µM) to eliminate NO. As reported, neither nitroarginine nor indomethacin (2.8 µM) affected the coronary vasodilator

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action of BK (10-1000 ng).

However, clotrimazole (1 μM), an inhibitor of P450 reduced responses up to 80% while 7-ethoxyresorufin, another P450 inhibitor, was less effective (40% inhibition). 17-ODYA (2 μM), an inhibitor of P450 fatty acid metabolism, also reduced responses to BK (up to 50%) suggesting a role of AA. None of the inhibitors affected responses to the reference vasodilator, nitroprusside (NP,1000 ng). Vasodilator responses to BK, but not NP, were markedly reduced by 10mM TEA (85%) and procaine (80%) suggesting an effect mediated by increased K^+ conductance. Nifedipine (5 nM) almost abolished responses to BK and cromakalim (1-10 μg) but did not affect those to NP. Inhibition of ATP-sensitive K^+ channels with glibenclamide (10 μM) reduced responses to cromakalim but not those to BK. These results suggest that the coronary vasodilator response to BK is mediated by a P450-AA metabolite that stimulates a Ca^{2+}-activated K^+ channel.

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CALPONIN PHOSPHORYLATION AND VASCULAR SMOOTH MUSCLE CONTRACTION

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In response to many agonists, both inositol(1,4,5)-triphosphate (InsP3) and diacylglycerol (DAG) are formed by the hydrolysis of an inositol lipid precursor stored in the plasma membrane of smooth muscle. The InsP3 released into the cytoplasm mobilizes calcium from internal stores, whereas DAG stimulates protein kinase C. Calponin is a thin filament-associated smooth muscle protein that has been implicated to play a role in the regulation of smooth muscle [1]. Recently, we found that smooth muscle calponin is an excellent substrate for protein kinase C and the phosphorylation reduced the binding of calponin to F-actin and tropomyosin [2-4]. We have identified an important phosphorylation site in calponin by protein kinase C and demonstrated the calponin phosphorylation response following stimulation by endothelin-1 or phorbol 12,13-dibutyrate (PDBu) in 32P-labeled porcine coronary artery [5]. We found that Thr184 is the preferred site of phosphorylation and is functionally the most important of the sites phosphorylated by protein kinase C in smooth muscle calponin. We investigated the calponin phosphorylation during endothelin-1 or PDBu stimulation of intact strips of porcine coronary artery. Stimulation by endothelin-1 or PDBu resulted in a significant increase of 32P incorporation into the calponin in association with development of force. These results suggest that calponin phosphorylation plays a potential role in the regulation of smooth muscle contraction by endothelin-1 or PDBu.

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INTERACTION OF NITROXIDERGIC NERVE WITH CHOLINERGIC AND NORADRENERGIC NERVES IN BLOOD VESSEL

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Nitroxidergic nerve in dog middle cerebral arteries originates from the pterygopalatine ganglion. VIPergic and cholinergic nerve cells and fibres are histochemically detected in the rat pterygoplatine ganglion which supplies nerve fibres to cerebral arteries. Nitroxidergic, cholinergic and VIPergic nerves are expected to be originated from the ganglion. Thus, we investigated functional the interrelationship between these nerves in isolated bovine basilar arteries. Transmural electrical stimulation (TES) relaxed the arteries. The response was not reduced in the VIP- and CGRP-tolerant arteries. Evidence for a mediation by NO of the response was obtained. The relaxation was reduced by acetylcholine or physostigmine and potentiated by atropine. VIP did not alter the response to TES. It is concluded that acetylcholine from cholinergic nerves appears to act on prejunctional muscarinic receptors and decrease the synthesis or release of NO in the nerve. VIP cannot be a transmitter nor a modulator of nitroxidergic nerve functions. In dog mesenteric arteries treated with α-adrenoceptor blocker, TES produced relaxation, which was proved to be mediated by NO. Treatment with EDRF or NO synthase inhibitors did not alter the release of norepinephrine by TES. These findings suggest that NO derived from the nerve does not interfere with the amine release from noradrenergic nerves.

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